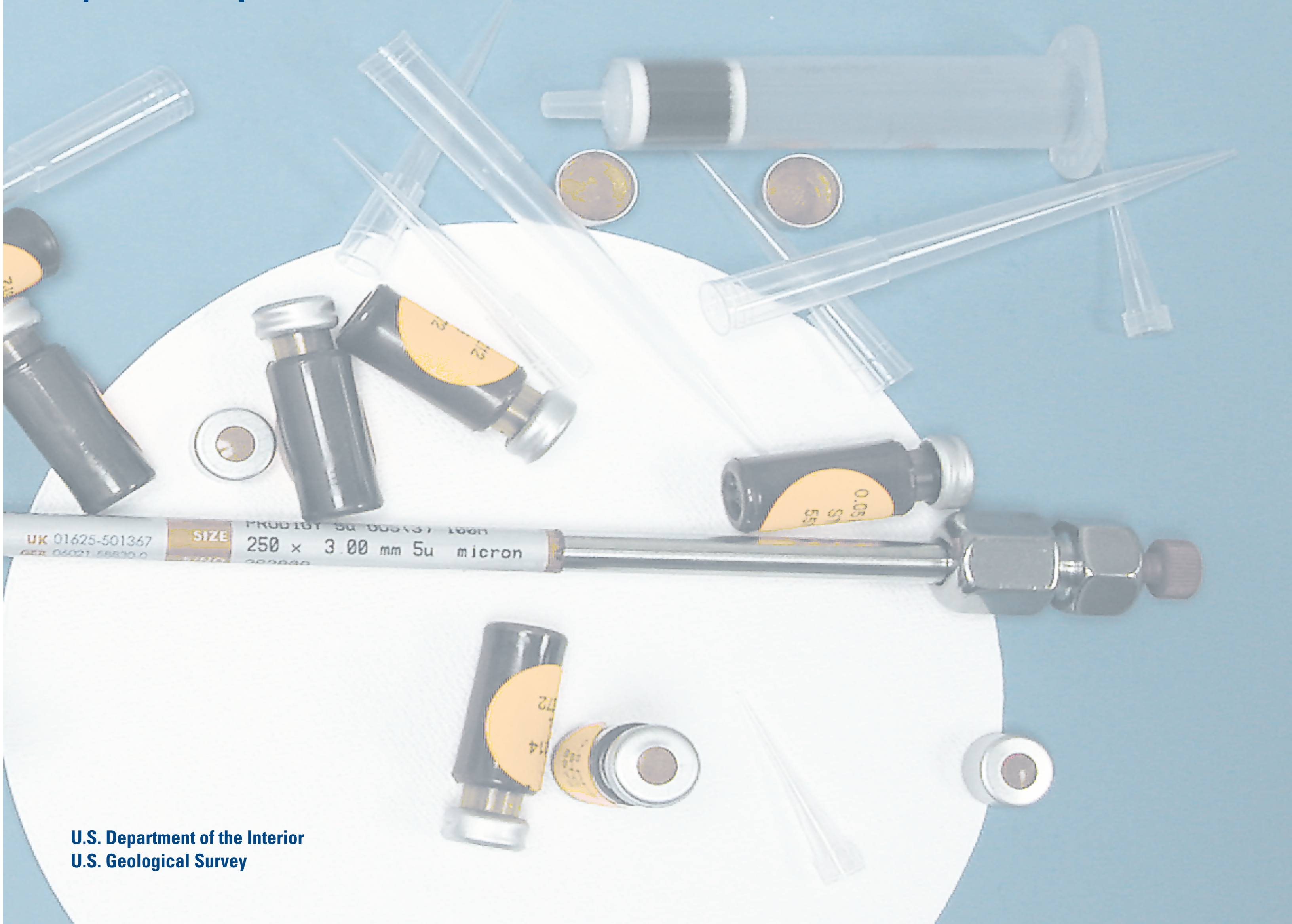
Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Triazine and Phenylurea Herbicides and Their Degradation Products in Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry

Open-File Report 02–436



U.S. Department of the Interior
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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **2002**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**
4. TITLE AND SUBTITLE **Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group--Determination of Triazine and Phenylurea Herbicides and Their Degradation Products in Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry**		5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Department of the Interior 1849 C Street, NW Washington, DC 20240**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release, distribution unlimited**		
13. SUPPLEMENTARY NOTES **The original document contains color images.**		
14. ABSTRACT		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**UU**	**26**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

U.S. Department of the Interior
U.S. Geological Survey

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By E.A. LEE, A.P. STRAHAN, and E.M. THURMAN

Open-File Report 02–436

Lawrence, Kansas
2002

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CONVERSION FACTORS, ABBREVIATED WATER-QUALITY UNITS, AND MISCELLANEOUS ABBREVIATIONS AND ACRONYMS

Conversion Factors

Multiply	By	To obtain
cubic centimeter (cm^3)	0.06102	cubic inch (in^3)
gram (g)	2.205×10^{-3}	pound (lb)
liter (L)	2.642×10^{-1}	gallon (gal)
meter (m)	3.281	foot (ft)
microgram per liter (µg/L)	1.0	part per billion (ppb)
microliter (µL)	2.642×10^{-7}	gallon (gal)
micrometer (µm)	3.937×10^{-5}	inch (in.)
micron (µ)	3.937×10^{-5}	inch (in.)
milligram (mg)	3.53×10^{-5}	ounce (oz)
milligram per liter (mg/L)	1.0	part per million (ppm)
millimeter (mm)	3.937×10^{-2}	inch (in.)
ounce (oz)	2.957×10^{-2}	liter (L)
pound per square inch (lb/in^2)	6.895	kilopascal (kPa)

Temperature can be converted to degrees Celsius (°C) or degrees Fahrenheit (°F) by the equations:

$$°C = 5/9\ (°F - 32),$$
$$°F = 9/5\ (°C) + 32.$$

Abbreviated Water-Quality Units

liter per minute (L/min)
microgram per liter (µg/L)
milligram per liter (mg/L)
milligram per milliliter (mg/mL)
milliliter (mL)
milliliter per minute (mL/min)
molar (M)

Miscellaneous Abbreviations and Acronyms

American Chemical Society (ACS)
Ångstrom (Å)
atomic mass unit (amu)
Chemical Abstracts Registry (CAS)
cubic centimeter (cm^3)
deethylatrazine (DEA)
deethyldeisopropylatrazine (DDA)
deisopropylatrazine (DIA)
deoxyribonucleic acid (DNA)
deuterated atrazine (D-5 atrazine)
diode array detector (DAD)
high-performance liquid chromatograph (HPLC)
liquid chromatograph (LC)
mass spectrometer (MS)
mass to charge (m/z)
Maximum Contaminant Level (MCL)
method detection limit (MDL)
minute (min)
mole (M)
octadecylsilane (C-18)
response factor (RF)
retention time (RT)
seconds (s)
solid-phase extraction (SPE)
U.S. Geological Survey (USGS)
volt (V)
volume per volume (v/v)
weight per volume (w/v)

Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Triazine and Phenylurea Herbicides and Their Degradation Products in Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry

By **E.A. Lee, A.P. Strahan,** *and* **E.M. Thurman**

Abstract

An analytical method for the determination of 7 triazine and phenylurea herbicides and 12 of their degradation products in natural water samples using solid-phase extraction and liquid chromatography/mass spectrometry is presented in this report. Special consideration was given during the development of the method to prevent the formation of degradation products during the analysis. Filtered water samples were analyzed using 0.5 gram graphitized carbon as the solid-phase extraction media followed by liquid chromatography/mass spectrometry. Three different water-sample matrices—ground-water, surface-water, and reagent-water samples—spiked at 0.2 and 2.0 micrograms per liter were analyzed.

Method detection limits ranged from 0.013 to 0.168 microgram per liter for the parent triazine herbicides and the triazine degradation products. Method detection limits ranged from 0.042 to 0.141 microgram per liter for the parent phenylurea herbicides and their degradation products. Mean recoveries for the triazine compounds in the ground- and surface-water samples generally ranged from 72.6 to 117.5 percent, but deethylcyanazine amide was recovered at 140.5 percent. Mean recoveries from the ground- and surface-water samples for the phenylurea compounds spiked at the 2.0-micrograms-per-liter level ranged from 82.1 to 114.4 percent. The mean recoveries for the phenylureas spiked at 0.2-microgram per liter were less consistent, ranging from 87.0 to 136.0 percent. Mean recoveries from reagent-water samples ranged from 87.0 to 109.5 percent for all compounds. The triazine compounds and their degradation products are reported in concentrations ranging from 0.05 to 2.0 micrograms per liter, with the exception of deethylcyanazine and deethylcyanazine amide which are reported at 0.20 to 2.0 micrograms per liter. The phenylurea compounds and their degradation products are reported in concentrations ranging from 0.20 to 2.0 micrograms per liter. The upper concentration limit was 2.0 micrograms per liter for all compounds without dilution.

INTRODUCTION

Triazine compounds are an important class of herbicides in the United States. Triazine herbicides such as atrazine and cyanazine are applied in the Midwestern United States for the control of weeds in corn, soybeans, and other row crops (Gianessi and Anderson, 1995). Atrazine has a Maximum Contaminant Level (MCL) of 3.0 μg/L and is the only triazine herbicide that is currently regulated under the Safe Drinking Water Act passed in 1974 (U.S. Environmental Protection Agency, 2002). Atrazine potentially causes the following health effects after humans are exposed at

concentrations greater than the MCL for relatively short periods of time: congestion of heart, lungs, and kidneys; low blood pressure; muscle spasms; weight loss; damage to adrenal glands. Long-term health effects may include cardiovascular damage, retinal and muscular degeneration, and cancer (U.S. Environmental Protection Agency, 2002).

Triazine compounds tend to degrade in the environment over time. Triazine half-lives are typically 30 to 60 days (Leonard, 1988). As the compounds degrade, new compounds such as atrazine derivatives and cyanazine acids are formed and persist in the environment. These degradation products may pose health effects for humans and animal life in the same way the parent products do. Recent studies have reported the occurrence of triazine degradation products and their importance. In some cases, as much as 81 percent of the total pesticide loads in the Iowa River in Iowa were pesticide degradation products (Schnoebelen and others, 2001). Deethylatrazine (DEA) and deisopropylatrazine (DIA) induce activity associated with endocrine disruption in adult male carp (Sanderson and others, 2001). Other studies have focused on the effects of parent compounds and some degradation products on animals such as rats. The atrazine degradation products deethyldeisopropylatrazine (DDA), DEA, DIA, and hydroxyatrazine did not cause gene mutation, chromosomal aberration, and deoxyribonucleic acid (DNA) damage in rats (Fan and Tomar, 1999).

Unfortunately, research on the effects of triazine degradation products on humans has been lacking. Some studies have been performed to determine if atrazine degradation products pose a threat to human and animal life. The current focus in humans has been to test for triazine degradation products to determine exposure to parent compounds such as atrazine. The human body processes atrazine in such a way that almost all is found as atrazine degradation products upon testing of bodily fluids (Fan and Tomar, 1999).

Phenylurea compounds are herbicides used for weed control. Phenylurea compounds such as diuron and linuron have relatively low acute toxicities in humans. However, they are irritants to the eyes, skin, and respiratory tract. Contamination of the aquatic environment is of more concern because diuron is toxic to fish and aquatic life at levels as low as 0.22 mg/L causing physiological and behavioral abnormalities (Pesticides News, 1994).

In understanding the fate and transport of parent herbicide compounds and their degradation products, reliable methods for the analysis of these compounds are vital. Reliable methods also are important for analytical verification of the degradation products in toxicological studies.

This report provides a detailed description of a method developed by the U.S. Geological Survey (USGS) Organic Geochemistry Research Group for the determination of 7 triazine and phenylurea herbicides and 12 of their degradation products in water. The description includes apparatus, reagents, instrument calibration, and solid-phase extraction (SPE) from ground-water, surface-water, and reagent-water samples. Method detection limits (MDLs), mean extraction recoveries, and relative standard deviations for the method also are presented.

Exposure to acid quickly begins the degradation of most triazine compounds. Therefore, care was taken to eliminate acid from the SPE and subsequent elution steps. The weak acid of the mobile phase was of concern, but it is in contact with each sample for only a short period of time (35 min).

Calibration and quantitation were accomplished using solutions of standard compounds processed through the entire method. This approach addressed losses of compounds during extraction and concentration.

The method of analysis described in this report has been assigned the USGS method number "O–2138–02." This unique code represents the automated method of analysis as it is described in the report and can be used to identify the method.

DETERMINATION OF TRIAZINE AND PHENYLUREA HERBICIDES AND THEIR DEGRADATION PRODUCTS IN WATER

Method of Analysis

Application

This method is suitable for the determination of low-level concentrations (in micrograms per liter) of the compounds listed in table 1 in ground- and surface-water samples. Because suspended particulate matter is removed from the samples by filtration, the method is suitable only for dissolved-phase compounds. The method may be suitable for other liquid samples such as wastewater, tile-drain effluents, and others matrices if they have been filtered; however, consideration

Table 1. Molecular weights and U.S. Geological Survey (USGS) parameter codes for triazine and phenylurea herbicides and their degradation products suitable for determination using method O–2138–02

[CAS, Chemical Abstracts Registry; T, triazine; P, phenylurea; --, not applicable]

Compound	CAS number	Molecular weight (atomic mass units)	USGS parameter codes	Herbicide type
Atrazine	1912–24–9	215.69	39632	T
Cyanazine	21725–46–2	240.70	04041	T
Cyanazine acid	--	259.70	61745	T
Cyanazine amide	--	258.70	61709	T
Deethylatrazine	6190–65–4	186.60	04040	T
Deethylcyanazine	--	212.64	61749	T
Deethylcyanazine acid	--	231.64	61750	T
Deethylcyanazine amide	--	230.66	61751	T
Deethyldeisopropylatrazine	3397–62–4	145.55	04039	T
Deethylhydroxyatrazine	--	169.18	62676	T
Deisopropylatrazine	1007–28–9	172.60	04038	T
Deisopropylhydroxyatrazine	--	155.16	62678	T
Demethylfluometuron	--	218.20	61755	P
Diuron	330–54–1	233.10	50374	P
Fluometuron	2164–17–2	232.20	38811	P
Hydroxyatrazine	2163–68–0	197.24	50355	T
Linuron	330–55–2	249.10	38478	P
Propazine	139–40–2	229.71	38535	T
Simazine	122–34–9	201.67	04035	T
Internal standard				
Simetone	673–04–1	197.00	--	T
Surrogates				
Chlorotoluron	1912–29–9	212.68	--	P
D-5 atrazine	15545–48–9	220.69	--	T

should be given to the fact that performance characteristics have not been assessed for these other liquid samples and that results have not been validated for these matrices.

Summary of Method

Water samples were filtered at the collection site using glass-fiber filters with nominal 0.7-µm pore diameter to remove suspended particulate matter. In the laboratory, the filtered water samples were spiked with the surrogate compounds and passed through a preconditioned graphitized carbon column. The carbon column was rinsed with reagent water to remove interfering substances. The absorbed compounds were eluted from the carbon column with a solution of methylene chloride, methanol, and ammonium hydroxide. The solution was spiked with an internal standard, evaporated under nitrogen, and reconstituted. The sample components were separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-performance liquid chromatograph (HPLC) equipped with a diode array detector (DAD) and a mass spectrometer (MS) detector operated in selected-ion monitoring mode. The concentrated sample solution was mixed with an acetic acid solution using an autosampler program immediately prior to injection into the LC/MS. Compounds eluting from the liquid chromatograph (LC) were identified by comparing the retention times of the mass spectral signals against the retention times of standards analyzed under the same conditions used for the samples. Compounds were identified further by selected fragment ions for compounds that produce fragment ions. The concentration of each identified compound was calculated by determining the ratio of the MS response produced by that compound to the MS response produced by the internal standard, which was injected into the sample, to the ratio of the MS responses of the primary standard analyzed using the same method. The molecular weights and USGS parameter codes for the compounds analyzed using method O–2138–02 are listed in table 1.

Interferences

Compounds that elute from the LC at the same times and have ions similar to the targeted compounds may interfere. Samples with high concentrations of

humic materials may cause interference with the ionization of the internal standard and the analyzed compounds if they elute from the LC at the same time.

Apparatus and Instrumentation

- *Analytical balances*—capable of accurately weighing 0.0100 g $\pm$0.0001 g.
- *Autopipettes*—5- to 500-µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, Massachusetts, or equivalent)
- *Tekmar six-position AutoTrace*—automated SPE workstation (Tekmar-Dohrmann, Cincinnati, Ohio, or equivalent).
- *Mechanical vortex mixer.*
- *Analytical column*—Luna (Phenomenex, Torrance, California) 250- x 3-mm, 5-µ particulate-size packing, pore size 100 Å, octadecylsilane (C-18).
- *HPLC/MS benchtop system*—Hewlett Packard (Wilmington, Delaware), model 1100 HPLC, with autosampler and MS detector.
  - LC column temperature conditions: constant 30 ^{o}C.
  - LC mobile-phase A: 0.1-percent acetic acid in 50/50 methanol/acetonitrile.
  - LC mobile-phase B: 0.1-percent acetic acid in reagent water.
  - LC flow rate: 0.400 mL/min.
  - LC gradient: 0 to 5 min 100-percent mobile-phase B; 5- to 30-min linear gradient to 100-percent mobile-phase A.
  - LC run time: 33 min, post run at 100-percent mobile-phase B, 6 min.
  - MS detector: atmospheric-pressure, chemical-ionization, positive-ion mode.
  - Drying gas flow: set at 7.0 L/min.
  - Nebulizer gas pressure: set at 30 lb/in^2.
  - Vaporizer temperature: set at 400 ^{o}C.
  - Gas temperature: set at 260 ^{o}C.
  - Fragmentor voltage: set at 100 V.
  - Capillary voltage: set at 2,000 V.
- *Data acquisition system*—computer and printer compatible with the HPLC system.
- *Software*—LC/MSD Chemstation revision 08.03 (Hewlett Packard, Wilmington, Delaware) was used to acquire and store data, for peak integration, and for quantitation of the compounds.

Reagents and Consumable Materials

- *Sample bottles*—baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—nominal 0.7-µm glass-fiber filters (Gilson, Middleton, Wisconsin, or equivalent).
- *0.1-mL autosampler vials*—plastic vial with glass-cone insert and cap (Wheaton, Millville, New Jersey).
- *SPE cartridges*—0.5-g graphitized carbon, 6 cm^3 (ENVI™-Carb 6-mL, Supelco, Bellefonte, Pennsylvania).
- *Analytical standards*—solutions of the herbicides and degradation products, the surrogates, and the internal standard.
- *Reagent water*—generated by purification of tapwater through activated charcoal filter and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in an autostill (Wheaton, Millville, New Jersey, or equivalent).
- *Disposable centrifuge tubes*—10 mL (Kimble, Vineland, New Jersey, or equivalent).
- *Solvents*—
  - Acetonitrile—American Chemical Society (ACS) and HPLC grade.
  - Methanol—ACS and HPLC grade.
  - Methylene chloride—ACS and HPLC grade.
- *Acetic acid, glacial*—ACS grade.
- *Ammonium hydroxide*—ACS grade.
- *Gas for evaporation*—nitrogen.
- *Pasteur pipettes*—(Kimble, Vineland, New Jersey, or equivalent).
- *Nebulizer*—nitrogen.

Sampling Methods

Sampling methods used were capable of collecting water samples that accurately represented the water-quality characteristics of the ground water or surface water at a given time or location. Detailed descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989). Detailed descriptions of sampling methods used by the USGS for obtaining depth- and width-integrated surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990).

Sample-collection equipment must be free of tubing, gaskets, and other components made of nonflu-

orinated plastic material that might leach interfering compounds into water samples or absorb the herbicides or degradation products from the water. The water samples from each site are composited in a single container and filtered through a nominal 0.7-µm glass-fiber filter using a peristaltic pump. Filters are preconditioned with about 200 mL of sample prior to filtration of the sample. The filtrate for analysis is collected in baked 125-mL amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory within 3 days of collection. At the laboratory, samples are logged in, assigned identification numbers, and refrigerated at 4 ^{o}C until extracted and analyzed.

Standards

- *Primary standard solutions*—Herbicide, degradation products, surrogates, and internal standard were obtained as pure material from commercial vendors or chemical manufacturers. Each was prepared at the concentration and in the solution listed in table 2.
- *Intermediate composite standards*—A 1.23-µg/mL composite standard was prepared by combining in a 500-mL volumetric flask appropriate volumes of the stock solution of the individual compounds. The composite solution was diluted with methanol and stored at less than 0 ^{o}C.
- *Internal standard solution*—The solution of simetone was prepared by diluting in a volumetric flask the appropriate amount to equal 0.123 mg/L using methanol.
- *Intermediate surrogate solution*—A 1.23-µg/mL composite solution of the surrogates was prepared from the stock solution of the individual compounds. The composite solution is prepared in methanol and stored at less than 0 ^{o}C.
- *Calibration standards*—At concentrations of 0.025, 0.05, 0.10, 0.20, 0.50, 1.00, and 2.00 µg/L, a series of calibration solutions is prepared in buffered reagent water (1.0 mL of 0.1 M phosphate buffer, pH 7.0, per 123 mL of distilled deionized water) using the intermediate composite standard solution.

Table 2. Stock solution composition for determination of triazine and phenylurea herbicides and their degradation products

[mg/mL, milligrams per milliliter]

Compound	Concentration (mg/mL)
Atrazine	1.000
Cyanazine	1.000
Cyanazine acid	2.340
Cyanazine amide	1.000
Deethylatrazine	.840
Deethylcyanazine	1.000
Deethycyanazine acid	1.010
Deethylcyanazine amide	1.010
Deethyldeisopropylatrazine	.109
Deethylhydroxyatrazine	.096
Deisopropylatrazine	1.040
Deisopropylhydroxyatrazine	.112
Demethylfluometuron	3.850
Diuron	1.000
Fluometuron	1.010
Hydroxyatrazine	.500
Linuron	1.000
Propazine	1.000
Simazine	
Internal standard	
Simetone	1.000
Surrogates	
Chlorotoluron	1.000
D-5 atrazine	1.000

Safety Precautions

- Perform all steps involving organic solvents and strong acids in a well-vented fume hood.
- Use appropriate personal protective equipment during the handling of any reagents and standards.
- The electrospray waste exhaust and the vacuum pump exhaust should be vented through a laboratory hood system.

Evaluation of Instrument Performance

High-Performance Liquid Chromatograph and Diode Array Detector Performance

HPLC performance is evaluated using background absorbance reading, peak shape, and system pressure.

Background absorbance signals should remain stable and low and indicate that the column has equilibrated with the mobile-phase flow. If peak shape deteriorates, the column may need to be replaced. If the pressure reading is high, there may be a clog in the mobile-phase flow path, or the column compartment thermostat may not have reached the required temperature. A variable DAD background signal indicates that the lamp may need to be replaced.

Mass Spectrometer Performance

The MS is tuned in atmospheric-pressure, chemical-ionization, positive-ion mode before each HPLC/MS analysis using the solutions, procedure, and software supplied by the manufacturer. With the first injection of each analysis, inject a solution of the mobile-phase solution to check for contamination.

Instrument Calibration

A calibration table and calibration curves were prepared for the analyzed standards using the LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware). This software uses the method and calculations as described in the alternate calibration listed in the following section. This includes the dilution correction factors that are entered as part of the sequence table used by the instrument to label and identify each injection. Manufacturer's instructions were followed for using the internal standard as time references and for quantitation.

Alternate Calibration

Data for each calibration point are acquired by injecting a mixture of 25 µL of extracted calibration solution plus 25 µL of 1.0-percent acetic acid into the HPLC/MS according to the conditions already described. The relative retention time is calculated for each selected compound in the calibration solution or in a sample as follows:

$$RRT_c = RT_c / Rt_i, \tag{1}$$

where

RRT_c = relative retention time,

RT_c = uncorrected retention time of the selected compound (minutes), and

RT_i = uncorrected retention time of the internal standard (minutes).

The results are presented in table 3.

- The expected retention time (RT) of the peak of the selected compound needs to be within ±2 percent of the expected retention time on the basis of the RRT_c obtained from the internal-standard analysis. The expected retention time is calculated using equation 2:

$$RT = (RRT_c)(RT_i), \tag{2}$$

where

RT = expected retention time of the selected compound (minutes),

RRT_c = relative retention time of the selected compound, and

RT_i = uncorrected retention time of the internal standard (minutes).

- The dilution factor of the processed sample is calculated using equation 3.

$$DF = \left(\frac{123}{123 - V_{np}}\right)\left(\left(\frac{123}{123 - V_a}\right), \tag{3}$$

where

DF = dilution factor,

V_{np} = volume not pumped (milliliters not pumped through the SPE column),

V_a = volume added (milliliters of distilled water added to a sample that contained less than 123 milliliters), and

123 = 123 milliliters of sample.

The dilution factor is incorporated into the calculation for determining final concentrations of samples.

- Initial calibration data are acceptable if the r^2 value for all curves is greater than or equal to 0.950 for all compounds.
- A complete extracted calibration curve is included within each instrument sequence.

Extraction Efficiency

Extraction efficiency is determined by analyzing the extracted 0.50-, 1.0-, and 2.0-µg/L standards against standards that were prepared for direct injection into the HPLC/MS. Both sets of standards were quantified using the internal standard method. The extrac-

Table 3. Retention times, relative retention times, molecular ions, and confirmation ions for triazine and phenylurea herbicides and their degradation products determined using method O–2138–02

[m/z, mass to charge; --, not applicable]

Compound	Retention time (minutes)	Relative retention time	Molecular ion (m/z)	Confirmation ion (m/z)
Atrazine	28.6	1.34	216	218
Cyanazine	26.0	1.21	241	243
Cyanazine acid	24.4	1.14	260	262
Cyanazine amide	21.6	1.01	259	261
Deethylatrazine	23.1	1.08	188	190
Deethylcyanazine	22.0	1.03	213	215
Deethylcyanazine acid	20.7	.97	232	234
Deethylcyanazine amide	18.3	.86	231	233
Dethyldeisopropylatrazine	14.8	.69	146	148
Deethylhydroxylatrazine	13.3	.62	170	--
Deisopropylatrazine	20.4	.95	174	176
Deisopropylhydroxyatrazine	6.7	.31	156	--
Demethylfluometuron	27.2	1.27	219	162
Diuron	29.0	1.36	233	235
Fluometuron	27.9	1.30	233	--
Hydroxyatrazine	17.0	.79	198	156
Linuron	30.7	1.43	249	251
Propazine	30.5	1.43	230	232
Simazine	26.4	1.23	202	204
Internal standard				
Simetone	21.4	1.00	198	--
Surrogates				
Chlorotoluron	28.1	1.31	213	215
D-5 atrazine	28.5	1.33	221	223

tion efficiency is the slope of the line obtained by plotting the value of the extracted standards calculated from the direct injection standards. The results are listed in table 4.

Solid-Phase Extraction Procedure

The SPE procedure used a Tekmar six-position AutoTrace (Tekmar-Dohrmann, Cincinnati, Ohio). The SPE columns used to extract samples were obtained from Supelco (Bellefonte, Pennsylvania). These vacuum cartridges contain 500 mg of graphitized carbon. The data in this report were produced using the Tekmar six-position AutoTrace procedure listed in Appendix 1.

- *Sample preparation*—123 mL is the volume that fits in the body of a 4-oz Boston round bottle. If an environmental sample contains less than 123 mL, distilled water is added to bring the volume to the required 123 mL. Any volume added is recorded. An extraction set consists of eight unknown samples, one duplicate sample, two standard samples, and a blank sample.
- *Adding surrogates*—100 µL of the surrogate intermediate solution is added to all blanks, standards, and samples.
- *Workstation preparation*—Before a sample set is extracted on the workstation, each port is flushed with 15 mL methanol/water (1:1) and then again with distilled water. All SPE columns, test tubes, reagents, and samples then are loaded onto the instrument.
- *Conditioning SPE columns*—The workstation conditions each SPE column by sequentially passing 8 mL methanol then 10 mL distilled water through each column at a flow rate of 15 mL/min by positive pressure.
- *Loading sample*—123 mL of each unknown, standard, and blank samples are passed through a SPE column at a flow rate of 10 mL/min.
- *Rinsing SPE column*—Each SPE column is rinsed with 5 mL distilled water at a flow rate of 20 mL/min.
- *Eluting compounds from SPE column*—Using the manual extraction manifold, each SPE column is eluted with 1 mL methanol followed by 6 mL of a solution of 45-percent methanol, 45-percent methylene chloride, and 10-percent ammonium hydroxide into a 10-mL disposable centrifuge tube. Each column then is eluted again with 7 mL of the solution of 45-percent methanol, 45-percent methylene chloride, and 10-percent ammonium hydroxide into another 10-mL disposable centrifuge tube. Concentration of eluting solution is prepared using volume-to-volume (v/v) measurements.

Table 4. Extraction efficiency of triazine and phenylurea herbicides and their degradation products in buffered reagent-water samples determined using method O–2138–02

Compound	Extraction efficiency (slope as a percentage)	Standard deviation (relative percentage)
Atrazine	93.1	16.0
Cyanazine	85.3	23.2
Cyanazine acid	93.6	21.4
Cyanazine amide	89.7	26.6
Deethylatrazine	94.1	14.3
Deethylcyanazine	77.0	25.3
Deethylcyanazine acid	82.7	18.8
Deethylcyanazine amide	91.4	33.6
Deethyldeisopropylatrazine	85.8	21.0
Deethylhydroxyatrazine	88.5	22.9
Deisopropylatrazine	94.6	16.7
Deisopropylhydroxyatrazine	130.3	45.9
Demethylfluometuron	87.9	23.9
Diuron	73.7	16.9
Fluometuron	85.4	11.0
Hydroxyatrazine	80.3	14.4
Linuron	104.2	42.4
Propazine	86.4	12.7
Simazine	86.1	16.5
Surrogates		
Chlorotoluron	91.6	9.1
D-5 atrazine	94.7	6.7
Mean	**90.0**	**22.3**
Maximum	**130.3**	**45.9**
Minimum	**73.7**	**6.7**

- *Spiking of internal standard*—After all the samples in a set have been eluted, the first tube of each elution is spiked with 500 µL of 0.123-mg/L simetone solution. The internal standard is used to normalize injection-volume variation, as a time reference, and for quantitation.
- *Evaporation*—The spiked solution then is evaporated under nitrogen in a water bath at 50 ^{o}C. The second elution of each sample then is transferred quantitatively to the first tube and again evaporated. Care is taken to remove the tubes from the evaporation apparatus immediately upon the tubes reaching dryness. The reconstitution step is performed immediately.
- *Reconstitution*—The extracts are reconstituted with 100 µL of a solution consisting of 50-percent methanol and 50-percent distilled water (v/v) and mixed thoroughly with a vortex mixer.
- *Transfer to vials*—Using a disposable Pasteur pipette, the reconstituted solution from the 10-mL glass centrifuge tube is transferred to an appropriately labeled autosampler vial containing a 0.1-mL insert for HPLC/MS analysis. The autosampler vial is capped and stored at less than 0 ^{o}C until analysis by HPLC/MS.
- *Sample analysis*—The HPLC/MS conditions for the analysis of the herbicides and their degradation products are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any sample extracts, the HPLC/MS is checked to verify that the performance criteria and the calibration data for herbicides and their degradation products conform to the criteria described. Immediately prior to injection, 25 µL of the sample extract are mixed with 25 µL of a solution of 1.0-percent acetic acid in water. The mixing is accomplished by programming the autosampler to perform that function. The mixed solution then is injected into the HPLC/MS.
- *Data acquisition*—The data are acquired using the Chemstation software.

Calculation of Results

Qualitative Identification

The LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware) is used with the previously prepared calibration table (table 3) for identification of compounds. A compound is not correctly identified unless it has the correct quantitation ion. If more than one ion is acquired for a compound, then additional verification is done by comparing the relative integrated abundance values of the significant ions monitored with relative integrated abundance values obtained from the standard samples. The relative ratios

of the ions need to be within ±20 percent of the relative ratios of those obtained from the standards.

Quantitation

The LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware) is used with the previously prepared calibration table (table 3) for quantification of the compound. This software allows for dilution factors to be entered and uses the internal standard for quantitation. Calibration curve fitting is by quadratic equation. Correlation coefficients should be 0.95 or greater.

Alternate Quantitation

If a selected compound has passed the qualitative identification criteria, the concentration in the sample is calculated as follows:

$$C = \left(\left(\frac{Ac}{Ai}\right)(m) + y\right)(DF), \text{ in micrograms per liter}, \quad (4)$$

where

- C = concentration of the selected compound in the sample, in micrograms per liter;
- Ac = area of peak of the quantitation ion for the selected compound;
- Ai = area of peak of the quantitation ion for the internal standard;
- m = slope of calibration curve using extracted standards between the selected compound and the internal standard from the original calibration data;
- y = intercept of calibration curve between the selected compound and the internal standard from the original calibration data; and
- DF = dilution factor calculated using equation 3.

Reporting of Results

The triazine herbicides and their degradation products are reported in concentrations ranging from 0.05 to 2.0 μg/L, with the exception of deethylcyanazine and deethylcyanazine amide which are reported at 0.20 to 2.0 μg/L. The phenylurea herbicides and their degradation products are reported to 0.20 to 2.0 μg/L. If the concentration is greater than 2.0 μg/L, the sample is re-extracted with a 1:10 dilution or greater (sample: distilled water) and re-analyzed for those compounds that have concentrations greater than 2.0 μg/L.

Method Performance

A buffered reagent-water sample, a ground-water sample collected from a well in Sedgwick County, Kansas, and a surface-water sample from the Kisco River below Mt. Kisco, New York, were used to test the performance of method O–2138–02. All samples were filtered through a nominal 0.7-μm glass-fiber filter and stored at 4 ^{o}C.

Subsamples of each sample matrix were spiked with the herbicides and degradation products listed in table 1 at concentrations of 0.2 and 2.0 μg/L and analyzed on different days from December 2001 through February 2002. In addition, unspiked subsamples of each sample matrix were analyzed. Comparisons of the different matrices and concentrations included bias from day-to-day variations. Method recoveries and standard deviations from the analyses are listed in tables 5–8.

Corrections for Background Concentrations

The unspiked subsamples of reagent water, ground water, and surface water from the Kisco River did not require correction for background concentrations.

Method Detection Limits

A method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDLs were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992). Eight replicate samples of buffered reagent water spiked with 0.025 μg/L of each of the triazine compounds and 0.20 μg/L of each of the phenylurea compounds were analyzed to determine MDLs (table 9). Each sample was analyzed on different days during December 2001 through February 2002 so that day-to-day variation is included in the results.

The MDL was calculated using the following equation:

$$MDL = (S)(t_{(\mathrm{n-1,\ 1-}\alpha\ =\ 0.99)}), \quad (5)$$

Table 5. Mean recovery and standard deviations for triazine and phenylurea herbicides and their degradation products in buffered reagent water analyzed using method O–2138–02

[µg/L, micrograms per liter]

Compound	Seven subsamples spiked at 0.2 µg/L				Seven subsamples spiked at 2.0 µg/L			
	Mean recovery		Standard deviation (µg/L)	Relative standard deviation (percent)	Mean recovery		Standard deviation (µg/L)	Relative standard deviation (percent)
	(µg/L)	(percent)			(µg/L)	(percent)		
Atrazine	0.198	99.0	0.021	10.6	1.958	97.9	0.047	2.4
Cyanazine	.197	98.5	.021	10.7	1.898	94.9	.225	11.3
Cyanazine acid	.200	100.0	.023	11.5	1.931	96.6	.298	14.9
Cyanazine amide	.200	100.0	.022	11.0	1.912	95.6	.218	10.9
Deethylatrazine	.195	97.5	.025	12.8	1.958	97.9	.084	4.2
Deethylcyanazine	.184	92.0	.016	8.7	1.883	94.2	.346	17.3
Deethylcyanazine acid	.174	87.0	.013	7.5	1.890	94.5	.232	11.6
Deethylcyanazine amide	.188	94.0	.023	12.2	1.912	95.6	.192	9.6
Deethyldeisopropylatrazine	.190	95.0	.037	19.5	2.013	100.7	.063	3.2
Deethylhydroxyatrazine	.199	99.5	.044	22.1	2.055	102.8	.198	9.9
Deisopropylatrazine	.188	94.0	.016	8.5	1.998	99.9	.066	3.3
Deisopropylhydroxyatrazine	.181	90.5	.044	24.3	2.032	101.6	.125	6.3
Demethylfluometuron	.200	100.0	.030	15.0	1.989	99.5	.188	9.4
Diuron	.195	97.5	.014	7.2	1.956	97.8	.149	7.5
Fluometuron	.197	98.5	.023	11.7	1.934	96.7	.122	6.1
Hydroxyatrazine	.180	90.0	.014	7.8	2.015	100.8	.101	5.1
Linuron	.219	109.5	.047	21.5	1.956	97.8	.149	7.5
Propazine	.203	101.5	.031	15.3	2.003	100.2	.089	4.5
Simazine	.198	99.0	.018	9.1	2.000	100.0	.113	5.7
Mean	**.194**	**97.0**	**.025**	**13.0**	**1.963**	**98.1**	**.158**	**7.9**
Minimum	**.174**	**87.0**	**.013**	**7.2**	**1.883**	**94.2**	**.047**	**2.4**
Maximum	**.219**	**109.5**	**.047**	**24.3**	**2.055**	**102.8**	**.346**	**17.3**
Surrogates								
Chlorotoluron (spiked at 1.0 µg/L)	1.053	105.3	.050	4.7	.907	90.7	.133	14.7
D-5 atrazine (spiked at 1.0 µg/L)	1.045	104.5	.091	8.7	.921	92.1	.149	16.2

Table 6. Mean recovery and standard deviations for triazine and phenylurea herbicides and their degradation products in ground water from a well in Sedgwick County, Kansas, analyzed using method O–2138–02

[μg/L, micrograms per liter]

Compound	Seven subsamples spiked at 0.2 μg/L				Seven subsamples spiked at 2.0 μg/L			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(μg/L)	(percent)	(μg/L)	(percent)	(μg/L)	(percent)	(μg/L)	(percent)
Atrazine	0.181	90.5	0.029	16.0	1.645	82.3	0.328	19.9
Cyanazine	.198	99.0	.038	19.2	1.786	89.3	.296	16.6
Cyanazine acid	.176	88.0	.030	17.0	1.516	75.8	.378	24.9
Cyanazine amide	.199	99.5	.027	13.6	1.818	90.9	.400	22.0
Deethylatrazine	.191	95.5	.020	10.5	1.804	90.2	.238	13.2
Deethylcyanazine	.231	115.5	.089	38.5	1.974	98.7	.383	19.4
Deethylcyanazine acid	.224	112.0	.010	4.5	1.755	87.8	.366	20.9
Deethylcyanazine amide	.281	140.5	.097	34.5	2.155	107.8	.662	30.7
Deethyldeisopropylatrazine	.221	110.5	.046	20.8	1.968	98.4	.315	16.0
Deethylhydroxyatrazine	.177	88.5	.061	34.5	1.917	95.9	.429	22.4
Deisopropylatrazine	.194	97.0	.021	10.8	1.870	93.5	.300	16.0
Deisopropylhydroxyatrazine	.173	86.5	.053	30.6	2.018	100.9	.466	23.1
Demethylfluometuron	.200	100.0	.036	18.0	1.673	83.7	.366	21.9
Diuron	.193	96.5	.036	18.7	1.641	82.1	.375	22.9
Fluometuron	.208	104.0	.034	16.3	1.693	84.7	.489	28.9
Hydroxyatrazine	.194	97.0	.037	19.1	1.803	90.2	.248	13.8
Linuron	.174	87.0	.182	104.6	1.752	87.6	.600	34.2
Propazine	.182	91.0	.033	18.1	1.725	86.3	.545	31.6
Simazine	.177	88.5	.033	18.6	1.629	81.5	.311	19.1
Mean	**.199**	**99.3**	**.048**	**24.4**	**1.797**	**89.8**	**.394**	**22.0**
Minimum	**.173**	**86.5**	**.010**	**4.5**	**1.516**	**75.8**	**.238**	**13.2**
Maximum	**.281**	**140.5**	**.182**	**104.6**	**2.155**	**107.8**	**.662**	**34.2**
			Surrogates					
Chlorotoluron (spiked at 1.0 μg/L)	.899	89.9	.157	17.5	.815	81.5	.220	27.0
D-5 atrazine (spiked at 1.0 μg/L)	.858	85.8	.131	15.3	.675	67.5	.071	10.5

Table 7. Mean recovery and standard deviations for triazine and phenylurea herbicides and their degradation products in surface water from Kisco River below Mt. Kisco, New York, analyzed using method O–2138–02

[µg/L, micrograms per liter]

Compound	Seven subsamples spiked at 0.2 µg/L				Seven subsamples spiked at 2.0 µg/L			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)
Atrazine	0.190	95.0	0.014	7.4	1.798	89.9	0.260	14.5
Cyanazine	.211	105.5	.046	21.8	1.826	91.3	.287	15.7
Cyanazine acid	.181	90.5	.029	16.0	1.451	72.6	.372	25.6
Cyanazine amide	.228	114.0	.059	25.9	1.786	89.3	.375	21.0
Deethylatrazine	.201	100.5	.043	21.4	1.759	88.0	.288	16.4
Deethylcyanazine	.235	117.5	.221	94.0	2.222	111.1	.636	28.6
Deethylcyanazine acid	.221	110.5	.043	19.5	1.739	87.0	.338	19.4
Deethylcyanazine amide	.224	112.0	.049	21.9	2.088	104.4	.698	33.4
Deethyldeisopropylatrazine	.222	111.0	.047	21.2	1.868	93.4	.204	10.9
Deethylhydroxyatrazine	.176	88.0	.072	40.9	1.934	96.7	.267	13.8
Deisopropylatrazine	.186	93.0	.023	12.4	1.867	93.4	.330	17.7
Deisopropylhydroxyatrazine	.153	76.5	.052	34.0	1.970	98.5	.310	15.7
Demethylfluometuron	.194	97.0	.041	21.1	1.820	91.0	.220	12.1
Diuron	.272	136.0	.140	51.5	1.789	89.5	.331	18.5
Fluometuron	.264	132.0	.076	28.8	1.725	86.3	.253	14.7
Hydroxyatrazine	.180	90.0	.028	15.6	1.727	86.4	.396	22.9
Linuron	.268	134.0	.235	87.7	2.288	114.4	.408	17.8
Propazine	.199	99.5	.021	10.6	1.867	93.4	.483	25.9
Simazine	.172	86.0	.023	13.4	1.761	88.1	.325	18.5
Mean	**.209**	**104.7**	**.066**	**29.7**	**1.857**	**92.9**	**.357**	**19.1**
Minimum	**.153**	**76.5**	**.014**	**7.4**	**1.451**	**72.6**	**.204**	**10.9**
Maximum	**.272**	**136.0**	**.235**	**94.0**	**2.288**	**114.4**	**.698**	**33.4**
Surrogates								
Chlorotoluron (spiked at 1.0 µg/L)	.964	96.4	.084	8.7	.754	75.4	.097	12.9
D-5 atrazine (spiked at 1.0 µg/L)	.927	92.7	.170	18.3	.757	75.7	.129	17.0

Table 8. Mean recovery and standard deviations for triazine and phenylurea herbicides and their degradation products in surface water from Clinton Lake, northeastern Kansas, analyzed using method O–2138–02

[µg/L, micrograms per liter]

Compound	Seven subsamples spiked at 0.2 µg/L					Seven subsamples spiked at 2.0 µg/L				
	Mean recovery					Mean recovery				
	Spiked subsample (µg/L)	Unspiked subsample (µg/L)	(percent)	Standard deviation (µg/L)	Relative standard deviation (percent)	Spiked subsample (µg/L)	Unspiked subsample (µg/L)	(percent)	Standard deviation (µg/L)	Relative standard deviation (percent)
Atrazine	0.772	0.572	100.0	0.134	17.4	2.017	0.572	72.3	0.374	18.5
Cyanazine	.211	--	105.5	.111	52.6	1.434	--	71.7	.255	17.8
Cyanazine acid	.170	--	85.0	.077	45.3	1.252	--	62.6	.373	29.8
Cyanazine amide	.153	--	76.5	.075	49.0	1.513	--	75.7	.194	12.8
Deethylatrazine	.373	.207	83.0	.102	27.3	1.813	.207	80.3	.298	16.4
Deethylcyanazine	.123	--	61.5	.124	100.8	1.839	--	92.0	.485	26.4
Deethylcyanazine acid	.221	--	110.5	.070	31.7	1.518	--	75.9	.233	15.3
Deethylcyanazine amide	.166	--	83.0	.174	104.8	1.886	--	94.3	.480	25.5
Deethyldeisopropylatrazine	.273	--	136.5	.096	35.2	1.832	--	91.6	.187	10.2
Deethylhydroxyatrazine	.238	--	119.0	.110	46.2	1.817	--	90.9	.223	12.3
Deisopropylatrazine	.253	.094	79.5	.064	25.3	1.657	.094	78.2	.225	13.6
Deisopropylhydroxyatrazine	.243	--	121.5	.099	40.7	1.821	--	91.1	.323	17.7
Demethylfluometuron	.190	--	95.0	.049	25.8	1.444	--	72.2	.306	21.2
Diuron	.212	--	106.0	.078	36.8	1.310	--	65.5	.349	26.6
Fluometuron	.197	--	98.5	.053	26.9	1.441	--	72.1	.355	24.6
Hydroxyatrazine	.630	.464	83.0	.187	29.7	2.068	.464	80.2	.421	20.4
Linuron	.084	--	42.0	.163	194.0	1.708	--	85.4	.470	27.5
Propazine	.170	.016	77.0	.029	17.1	1.538	.016	76.1	.598	38.9
Simazine	.179	--	89.5	.042	23.5	1.462	--	73.1	.224	15.3
Mean	**.175**	**--**	**92.2**	**.097**	**49.0**	**1.501**	**--**	**79.0**	**.335**	**20.6**
Minimum	**.084**	**--**	**42.0**	**.029**	**17.1**	**1.252**	**--**	**62.6**	**.187**	**10.2**
Maximum	**.772**	**--**	**136.5**	**.187**	**194.0**	**2.068**	**--**	**94.3**	**.598**	**38.9**
Surrogates										
Chlorotoluron (spiked at 1.0 µg/L)	.828	--	82.8	.146	17.6	.688	--	68.8	.200	29.1
D-5 atrazine (spiked at 1.0 µg/L)	.792	--	79.2	.115	14.5	.621	--	62.1	.088	14.2

Table 9. Mean concentrations and method detection limits for eight determinations of triazine and phenylurea herbicides and their degradation products in eight samples of buffered reagent water analyzed using method O–2138–02

[μg/L, micrograms per liter]

Compound	Spiked level (μg/L)	Mean concentration (μg/L)	Standard deviation (μg/L)	Method detection limit (μg/L)
Atrazine	0.025	0.0340	0.0117	0.035
Cyanazine	.025	.0314	.0044	.013
Cyanazine acid	.025	.0270	.0056	.017
Cyanazine amide	.025	.0256	.0058	.017
Deethylatrazine	.025	.0290	.0048	.015
Deethylcyanazine	.20	.1650	.0560	.168
Deethylcyanazine acid	.025	.0268	.0102	.031
Deethylcyanazine amide	.025	.0290	.0188	.057
Deethyldeisopropylatrazine	.025	.0340	.0116	.035
Deethylhydroxyatrazine	.025	.0294	.0067	.020
Deisopropylatrazine	.025	.0176	.0056	.017
Deisopropylhydroxyatrazine	.025	.0280	.0081	.024
Demethylfluometuron	.200	.200	.030	.090
Diuron	.200	.195	.014	.042
Fluometuron	.20	.197	.023	.069
Hydroxyatrazine	.025	.0310	.0053	.016
Linuron	.20	.219	.047	.141
Propazine	.025	.0296	.0067	.020
Simazine	.025	.0324	.0081	.024

where

S = standard deviation of replicate analysis, in micrograms per liter, at the spiked concentration;

$t_{(n-1,\ 1-\alpha,\ =\ 0.99)}$ = Student's t-value for the 99-percent confidence level with n-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and

n = number of replicate analyses.

The estimated MDL for each compound is listed in table 9. Method detection limits ranged from 0.013 to 0.168 μg/L for the triazine compounds and from 0.042 to 0.141 μg/L for the phenylurea compounds. According to the U.S. Environmental Protection Agency (1992) procedure, the spiked concentrations should be no more than five times the estimated MDL. The spiked concentrations were within five times the MDL for the method analysis described in this report.

Mean Recovery

Mean recoveries in reagent-water, ground-water, and surface-water samples were determined by comparing the mean analyzed concentration (see "Quantitation" section) from the eight replicate samples to the spiked concentration. The mean recoveries of all water samples spiked at 0.2 μg/L ranged from 76.5 to 140.5 percent and from 72.6 to 114.4 for all water samples spiked at 2.0 μg/L. Mean recoveries in reagent-water samples ranged from 87.0 to 109.5 percent with a mean of 97.6 percent for all compounds (table 5). Mean recoveries for the triazine compounds in the ground- and surface-water samples ranged from 72.6 to 115.5 percent with deethylcyanazine amide recovered at 140.5 percent. Both percentage extremes were from the samples spiked at 0.2 μg/L.

DISCUSSION

The basic premise for developing method O–2138–02 was to have a reliable analytical method that prevented degradation of triazine compounds by exposure to acid during the isolation and concentration of the sample and to improve upon the sensitivity of the analyses. This is necessary to accurately analyze for the parent herbicides but increases in importance with the inclusion in the method of many degradation compounds of the triazine herbicides.

During the degradation process, many different triazine herbicides form identical chemical compounds (Scribner and others, 2000). The degradation products of cyanazine, deethylcyanazine and deethylcyanazine amide, are characterized by high recoveries and high relative standard deviations in ground- and surface-water samples spiked at 2.0 μg/L—recoveries range from 110.5 to 140.5 percent and relative standard deviations from 19.5 to 94.0 percent. However, recoveries and relative standard deviations from the reagent-water

samples do not reflect this trend. This indicates that the matrices of the ground- and surface-water samples may be degrading the cyanazine herbicide and its degradation products after spiking but before extraction. The same relation is found, but to a much lesser extent, in the 2.0-μg/L spiked samples.

Method O–2138–02 is not optimized for sensitivity to the phenylurea compounds but yields reliable results within the listed MDLs. Water matrices play an important role with the phenylurea compounds that result in greater variations from one matrix to another as compared to the triazine compounds.

Care was taken to use an internal standard, simetone, another triazine compound, that is in the same chemical class. The use of deuterated atrazine (D-5 atrazine), which reacts chemically identical to atrazine but has a different molecular mass (+5 amu), as a surrogate standard allows for monitoring of the entire method. Chlorotoluron is used as the surrogate for the phenylurea compounds for the same reason.

Figure 1, the total ion chromatogram of a 1.0-μg/L standard in a buffered reagent-water sample, shows the separation of the compounds by method O–2138–02. Although some compounds co-elute, they are differentiated by the mass spectrometer. The co-eluting compounds have different molecular weights and different confirmation ions.

A very difficult matrix to recover herbicide compounds from, based on experience in the USGS Organic Geochemistry Research Group laboratory, is a midwinter sample from Clinton Lake in northeastern Kansas. A sample from Clinton Lake was included in the analyses by method O–2138–02 to demonstrate possible recoveries and standard deviations in a difficult matrix. Spiked concentrations in the samples from Clinton Lake were corrected for background concentrations of atrazine (0.572 μg/L), deethylatrazine (0.207 μg/L), deisopropylatrazine (0.094 μg/L), hydroxyatrazine (0.464 μg/L), and propazine (0.016 μg/L). The results are listed in table 8. Nine of the nineteen compounds had either low recoveries or large relative standard deviations for the sample spiked at 0.2 μg/L.

CONCLUSIONS

Method O–2138–02 provides for routine analyses of 7 triazine and phenylurea herbicides and 12 of their degradation products and guards against the formation of degradation products during the performance of the method. The method demonstrates that SPE with graphitized carbon coupled with liquid chromatography/mass spectrometry can be used to analyze water samples for the listed compounds. Good precision and accuracy for the analysis of compounds were shown for reagent water, ground water, and surface water with the exception of linuron spiked at 0.2 μg/L. Method detection limits ranged from 0.013 to 0.168 μg/L for the triazine compounds and ranged from 0.042 to 0.141 μg/L for the phenylurea compounds. The mean recoveries of all water samples spiked at 0.2 μg/L ranged from 76.5 to 140.5 percent and from 72.6 to 114.4 percent

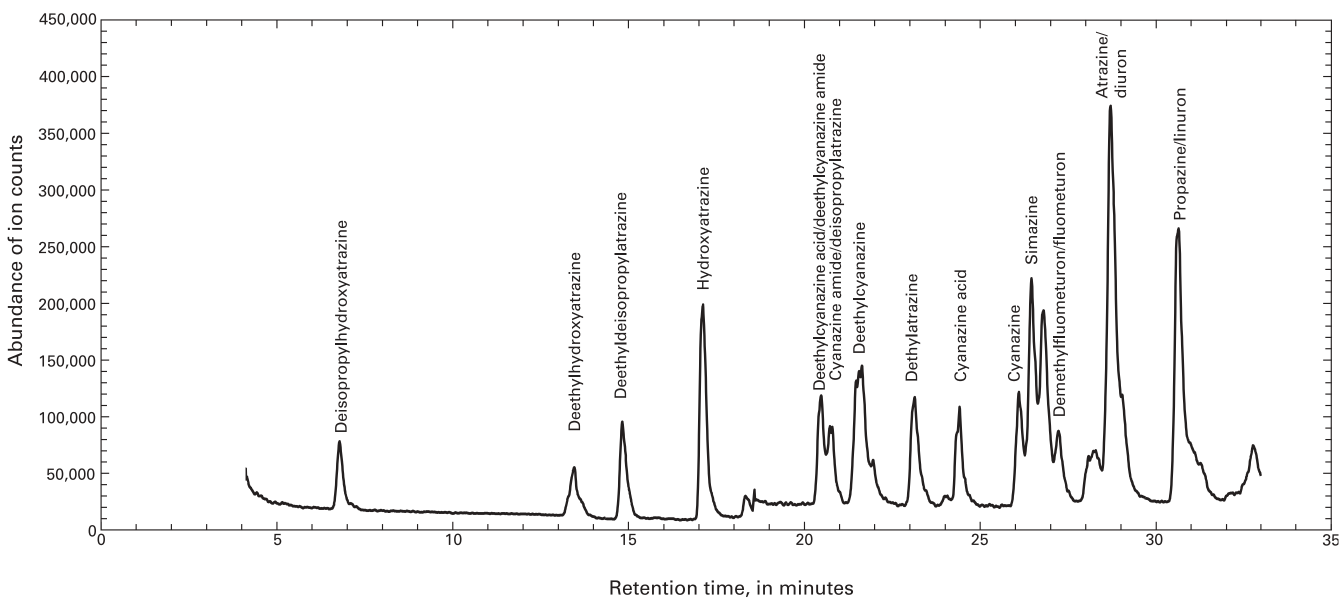

Figure 1. Total ion chromatogram of 1.0-microgram-per-liter standard in buffered reagent water using method O–2138–02.

for all water samples spiked at the 2.0 μg/L. The triazine herbicides and their degradation products are reported in concentrations ranging from 0.05 to 2.0 μg/L, with the exception of deethylcyanazine and deethylcyanazine amide which are reported at 0.20 to 2.0 μg/L. The phenylurea herbicides and degradation product were reported in concentrations ranging from 0.20 to 2.0 μg/L. The upper concentration limit was 2.0 μg/L for all compounds without dilution.

Information about the fate and transport of triazine and phenylurea herbicides and their degradation products in water can be acquired from the analysis of ground water and surface water using method O–2138–02. This method also can be used for water-quality determinations.

REFERENCES CITED

Edwards, T.K., and Glysson, G.D., 1988, Field methods for measurement of fluvial sediment: U.S. Geological Survey Open-File Report 86–531, 118 p.

Fan, A., and Tomar, R., 1999, Public health goal for atrazine in drinking water: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, February 1999, p. 1–45.

Gianessi, L.P., and Anderson, J.E., 1995, Pesticide use in U.S. crop production—national data report: Washington, D.C., National Center for Food and Agricultural Policy, unnumbered pages.

Hardy, M.A., Leahy, P.P., and Alley, W.M., 1989, Well installation documentation and ground-water sampling protocols for the pilot National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 89–396, 36 p.

Leonard, R.A., 1988, Herbicides in surface water, *in* Grover, R. ed., Environmental chemistry of herbicides—volume I: Boca Raton, Florida, CRC Press, p. 45–88.

Pesticide News, 1994, Misguided herbicides: v. 24, June 1994, p. 16–17.

Sanderson, J.T., Letcher, R.J., Heneweer, M., Giesy, J.P., and van den Berg, M., 2001, Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp *Hepatocytes*: Environmental Health Perspectives, v. 109, no. 10, p. 1027–1031.

Schnoebelen, D.J., Kalkhoff, S.J., and Becher, K.D., 2001, Occurrence and distribution of pesticides in streams of the Eastern Iowa Basins, 1996–98, *in* Proceedings of Agriculture and the Environment—State and Federal Initiatives Conference, May 5–7, 2001: Ames, Iowa State University, p. 85–86.

Scribner, E.A., Thurman, E.M., and Zimmerman, L.R., 2000, Analysis of selected herbicide metabolites in surface and ground water of the United States: Science of the Total Environment, v. 248, nos. 2–3, p. 157–167.

U.S. Environmental Protection Agency, 1992, Guidelines establishing test procedures for the analysis of pollutants—appendix B, part 136, Definition and procedures for the determination of the method detection limit: U.S. Code of Federal Regulations, Title 40, revised as of July 1, 1992, p. 565–567.

———2002, Consumer factsheet on atrazine: Information available on the World Wide Web, accessed April 5, 2002, at URL www.epa.gov/safewater/dwh/c-soc/atrazine.html

Ward, J.R., and Harr, C.A., 1990, Methods for collection and processing of surface-water and bed-material samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90–140, 71 p.

Appendix

APPENDIX 1. AUTOTRACE PROGRAM

AutoTrace extraction procedure: method O–2138–02
Estimated time for samples: 32.4 min

Step 1 :Process six samples using the following steps.
Step 2 :Condition column with 8 mL methanol into SOLVENT WASTE.
Step 3 :Condition column with 10 mL deionized water to AQUEOUS WASTE.
Step 4 :Load 127 mL of sample onto column.
Step 5:Rinse column with 5 mL deionized water into AQUEOUS WASTE.
Step 6 :END.

SETUP PARAMETERS

Flow rates

Condition flow:15.0 mL/min
Load flow:10.0 mL/min
Rinse flow:20.0 mL/min
Elute flow:5.0 mL/min
Condition air push:15.0 mL/min
Rinse air push:20.0 mL/min
Elute air push:5.0 mL/min

SPE parameters

Push delay:5 s
Air factor:1
Autowash volume:1.00 mL

Workstation parameters

Maximum elution volume:12.0 mL
Exhaust fan on:Yes
Beeper on:Yes

Name solvents

Solvent 1:none
Solvent 2:Methanol
Solvent 3:Deionized water
Solvent 4:none
Solvent 5:none